GENETICS

Genetic control of regeneration processes of radish plants *in vitro*: from phenotype to genotype

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Abstract

This review highlights the years of research on the genetics of in vitro regeneration in higher plants conducted at the Department of Genetics and Biotechnology of Saint Petersburg State University. The genetic collection of radish (*Raphanus sativus*) created at the department by selfing of individual plants from three cultivars was used as a model in these studies. Some radish inbred lines from the genetic collection form spontaneous tumors in the roots and are also used to study mechanisms of tumor growth in higher plants. It was revealed that radish lines that differed in the ability to form tumors also contrastingly differed in the reaction of their explants to auxin and cytokinin in vitro, which reflects a difference in the levels of these hormones in the tissues of related tumorous and non-tumorous radish lines. Moreover, high concentrations of cytokinins in cultural medium induced tumor formation in the regenerated plants of tumorous radish lines. The presence of meristematic zones in spontaneous tumors in radish lines, as well as in crown gall tumors induced by *Agrobacterium tumefaciens* and cytokinin-induced tumors made it possible to reveal the role of the main meristem regulators, such as KNOX and WOX family transcription factors and the CLAVATA system, in both the process of tumor growth and regeneration in plants. Analysis of the expression of meristem-specific genes during the development of spontaneous and induced tumors in radish as well as in regenerated radish plants confirmed this assumption.

Keywords: *Raphanus sativus*, regeneration, tumors, meristems, phytohormones.

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Introduction

In 1969, at the Department of Genetics and Breeding (now the Department of Genetics and Biotechnology), a research group was organized to develop methodologies for working with plant cells and tissues culture in vitro. As a result, by the 1970s, a new direction emerged to study the genetics of plant regeneration as a model of differentiation and organogenesis processes.

This work continues to this day, but using the methodology of reverse genetics. Currently, the main goal of our research is to identify conservative mechanisms for maintaining stem cell pools in higher plants. Stem cells are undifferentiated cells of multicellular organisms capable of dividing, self-renewing and differentiating. Despite the existing differences in properties, general principles of stem cell existence can be distinguished in all multicellular organisms (reviewed in Dodueva et al., 2017). In plants, stem cells are found in meristems — structures that ensure continuous plant growth and provide material for the formation of various specialized tissues. There are several types of meristem: shoot and root apical meristems, lateral meristems (procambium, cambium, pericycle), and also so-called irregular meristems, developing under certain conditions (callus, meri-

stems of symbiotic nodules, spontaneous and pathogen-induced tumors, etc.).

Due to the activity of meristems, plants retain their ability to grow indefinitely. In addition, due to their immobility, plants are constantly exposed to various adverse effects of the environment, both biological and non-biological, and, probably because of this, one of the main evolutionary "acquisitions" of higher plants that ensured their successful survival is their unique ability to regenerate.

The term "regeneration" in relation to plants is used by different authors in different ways: for example, it can mean only the restoration of a lost part or its replacement by callus. In other cases, this term, in addition to the above processes, may also include some variants of vegetative propagation. However, the cause that triggers regeneration is usually damage of plant organs or tissues (wounding) (Perez-Garcia and Moreno-Risueno, 2018).

The development of methods of plant in vitro cultivation by the middle of the 20th century expanded the concept of regeneration and led to the emergence of a number of new phenomena that did not previously exist in nature: for example, callus formation from explants, somatic embryogenesis from callus, and regeneration via shoot and root formation. A number of in vitro plant regeneration protocols have been developed (and are still being developed), including the production of sterile material, cultivation on specially selected media with a certain ratio of mineral substances and growth regulators, etc. The in vitro regeneration phenomena made it possible to more deeply uncover a unique feature of plants: the presence of cells capable of dedifferentiation, gaining totipotency and further differentiation, and also finding many external regulators capable of influencing these processes. At the same time, about the internal regulation of the processes of callus formation and plant regeneration, in particular, about their molecular genetic control, at that time almost nothing was known (Fadeeva et al., 1979).

In vitro regenerative capacity on the example of radish inbred lines

A number of studies confirm the similarity of the processes occurring during callusogenesis and plant regeneration in vitro with the development of different types of meristem (Atta et al., 2009; Sugimoto et al., 2010). Thus, regenerating cell cultures can be used as a model for studying the genetic control of plant meristem development. The main goal of studying the genetic mechanisms of plant regeneration was the search for genes that determine the ability to "start all over again", in which the unique property of plants — the totipotency of any cells — is fully realized.

The mechanisms of the totipotency of plant cells and genetic control of plant in vitro regeneration have become the subject of many years of research at the Department of Genetics and Biotechnology of Saint Petersburg State University. The preliminary stage of work consisted of identifying genotypic diversity of the ability to regenerate in vitro, unfolding the complex trait "regeneration" into simple ones, and studying their nature and inheritance. The objects in these studies were genetic collections of agricultural plant species such as radish, pea, potato, and strawberry, —species with different reproduction biology (Fadeeva et al., 1979). In this review, we will focus on the genetic collection of radish (*Raphanus sativus*), which was created in 1960 by selfing individual plants of three cultivars of different origin (Narbut, 1966). The collection currently contains 33 inbred lines, many of which are characterized by impaired morphogenesis (stem bends, decreased apical dominance, premature seed germination, dwarfism, etc.) (Buzovkina and Lutova, 2007). One of the developmental anomalies in radish inbred lines is the spontaneous formation of tumors on the root during flowering (Narbut, 1967). The genetic collection now includes ten tumorous inbred lines, which are found among the lines originating from all three cultivars (Narbut et al., 1995; Buzovkina and Lutova, 2007).

Evaluation of the regenerative capacity of radish inbred lines and cultivars in the in vitro system revealed their differences in this trait (Fadeeva et al., 1975; Lutova et al., 1994; Lutova et al., 1997) (Fig. 1). The essence of our approach was to divide such a complex and diverse phenomenon as regeneration into elementary traits (callus, root and shoot formation from explants). Testing the ability to regenerate was carried out at the level of primary explants (cotyledons, parts of the stem), which are close in their properties to the plant, in contrast to the passaged calli, which often lose the properties of the original genotype due to somaclonal variability (Lutova et al., 1994). Numerous studies have shown that radish cultivars have similar type and optimal response to damage — a high ability of callus and root formation (adaptive traits), which can be designated as a universal response to wounding. This mechanism was probably developed in plant evolution and provides versatility in maintaining a certain hormonal status, which ensures a high regenerative capacity. On the other hand, in general, inbred lines had a lower level of callus, shoot and root formation than the original cultivars, and hybrids of inbred lines restored the ability to regenerate to the level of cultivars. A number of genes responsible for the explants' ability of callus formation and root formation have been identified by genetic analysis in the radish (Lutova et al., 1994).

Features of in vitro regeneration in radishes are often correlated with the ability of tumor formation in a given line. Thus, explants of tumorous radish lines formed callus during regeneration, but did not form roots (Lutova

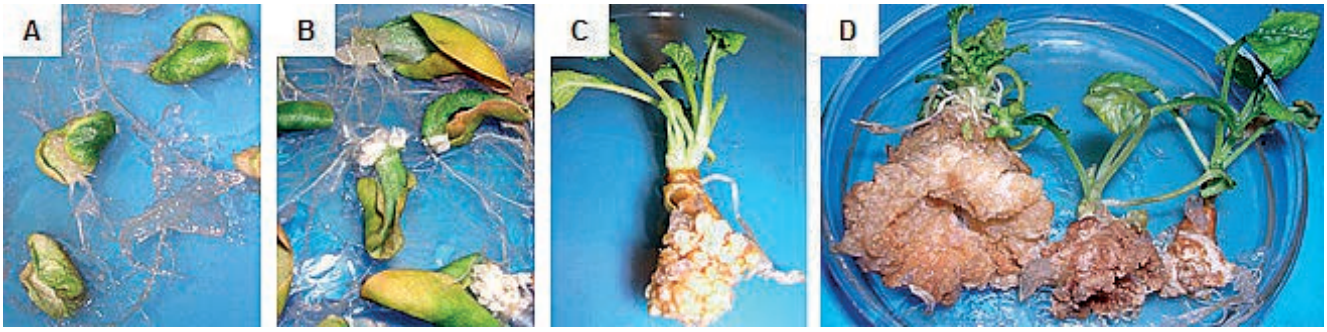

Fig. 1. Regeneration traits signs in radish explants: A — callus formation in cotyledon explants, B — root formation in cotyledon explants; C — crop-root-like structure; D — crop-root-like structures with tumors.

et al., 1988; Lutova et al., 1997). Such observations indirectly confirmed the presence of common mechanisms for regulating cell proliferation and differentiation in the in vitro and in vivo systems.

It is important to mention that plant regeneration in vitro is usually achieved by adding exogenous phytohormones, especially auxins and cytokinins. Thus, differences in the regeneration traits in different forms often indicate a different content and ratio of endogenous phytohormones (Lutova et al., 1994).

Radish lines demonstrated the relationship between the reaction to exogenous hormones, the traits of regeneration and the ability of tumor formation. Thus, it was shown that tumor-forming radish lines, unlike non-tumorous, are characterized by increased sensitivity to exogenous auxins and/or cytokinins during cultivation in vitro, which probably indicates an increase in the level of endogenous phytohormones in the tissues of these lines or cellular response to them, which apparently leads to in vivo tumor formation (Buzovkina et al., 1993a; Matveeva et al., 2000). Moreover, for all tumorous radish lines but none of the non-tumorous lines, hormone-independent cell cultures capable of supporting proliferation on hormone-free media were obtained (Buzovkina et al., 1993b).

Later, a protocol was developed for obtaining so-called crop-root-like structures by cultivating the apexes of aseptic radish plants on medium with cytokinins (Buzovkina et al., 1993b). In the lower part of the hypocotyls of plants regenerated form apexes on cytokinin-rich medium, structures were developed that were externally and anatomically similar to the crop-roots which radish forms in vivo (crop-roots are storage organs consisting of secondary thickened root and hypocotyl) (Buzovkina et al., 1993b; Ilyina et al., 2006) (Fig. 1). Crop-root-like structures obtained on the regenerated plants of the tumorous radish lines later formed tumors capable of hormone-independent growth. So, it is possible to simulate spontaneous tumor formation in radish in the in vitro system, and this approach greatly simplified work with radish tumors (Buzovkina et al., 1993b). These cytokinin-induced tumors are also anatomically similar to the tumors formed on the crop-roots of tumorous radish lines in the field and are formed as a result of extensive cell proliferation in the lateral meristems — the pericycle and cambium (Ilyina et al., 2006; Lebedeva et al., 2015) (Fig. 2).

The processes of dedifferentiation and secondary differentiation take place not only during organ explantation in vitro, but also as a result of plant transformation with *Agrobacterium*. Our research has shown that, as a rule, high ability of radish lines and cultivars of agrobacterial transformation correlated with high capacity for regeneration (Lutova et al., 1994). Later, these assumptions were confirmed in the works of foreign researchers: cells competent for transformation by *Agrobacterium* are also competent for regeneration — as a rule, these are cells originated from the pericycle (Azmi et al., 2000; Sugimoto et al., 2011).

Thus, the endogenous balance of phytohormones is the basis of the processes of plant cell division and differentiation. For certain genotypes, in fact, a minor impact on the plant (wounding, infection with agrobacteria, increased concentration of cytokinins) is enough to begin cell division followed by differentiation. It follows from the obtained results that the endogenous balance of phytohormones and its lability may be one of the mechanisms of ontogenetic adaptation of higher plants.

Tumor growth is a model for studying the processes of plant cell division and differentiation

Why did spontaneous tumor formation in the inbred lines of radish genetic collection, and then other types of tumors, become the objects of research at the Department of Genetics and Biotechnology? Examples of tumor growth in higher plants are quite rare — perhaps this is due to the redundancy of genes acting at each stage of the systemic control of cell division in plants (Doonan

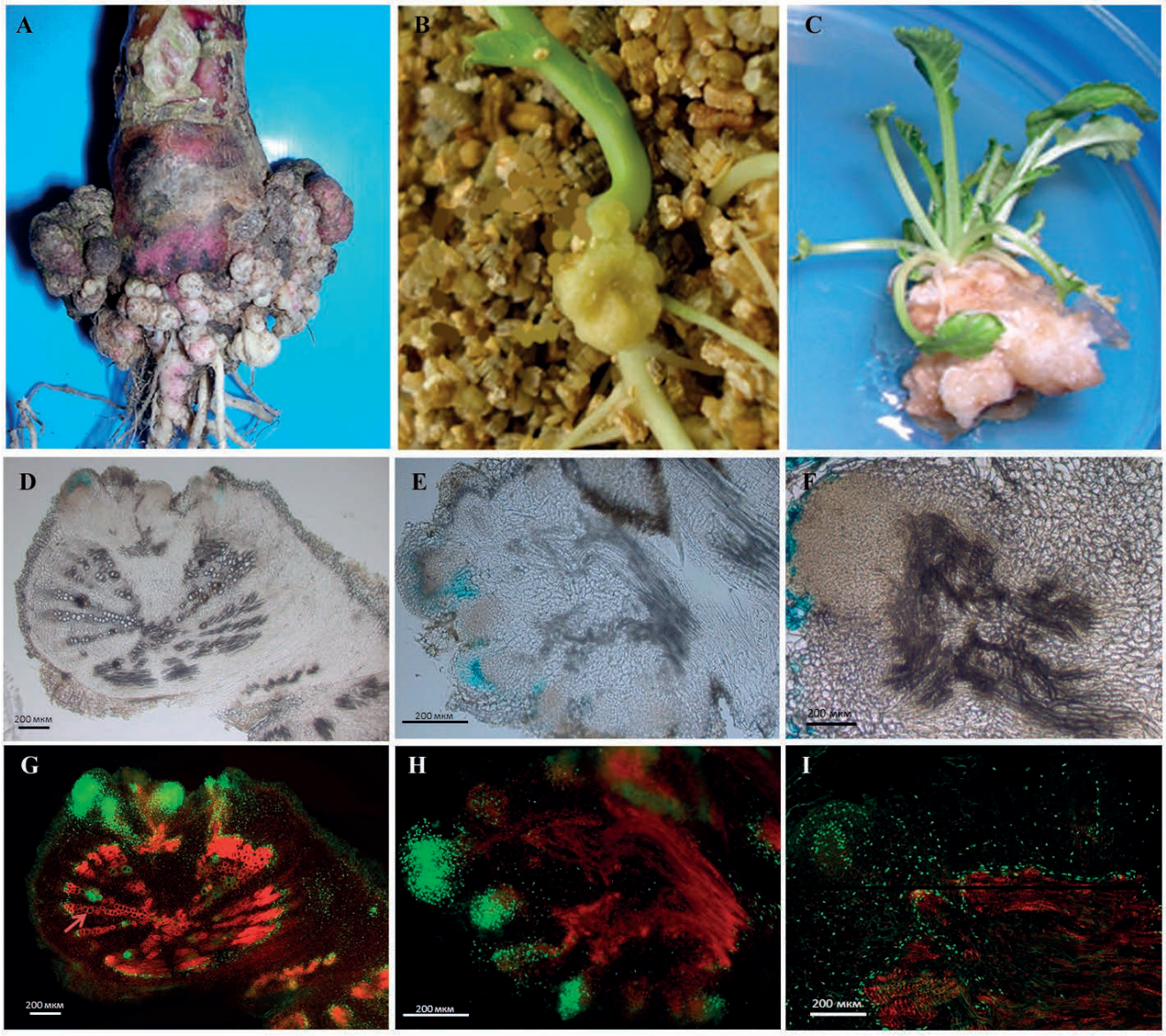

Fig. 2. Anatomy of tumors of different origin in plants: A, D, G — spontaneous tumor on the root of radish line 19; B, E, H — tumor induced on radish hypocotyl by *Agrobacterium tumefaciens*; C, F, I — tumor induced by cytokinin (BAP, 2 mg / l) in radish regenerated plant *in* vitro. A–C — appearance of tumors; D–F — cross-sections staining with toluidine blue; G–I — detection of proliferating cells by EdU-Alexa Fluor488 (green).

and Hunt, 1996). So, tumor formation in higher plants is a suitable model for studying key mechanisms of systemic control. In various plant species, there are quite a few examples of the development of galls (neoplasms with limited growth) and tumors (neoplasms capable of unlimited cell proliferation even when removed from the mother plant and a large number of passages on the hormone-free medium) under the influence of various parasitic organisms (bacteria, fungi, nematodes, arthropods) (Dodueva et al., 2007; Dodueva and Lutova, 2011). As a rule, the formation of such pathogen-induced neoplasms is associated with the local activation of phytohormone (auxins, cytokinins, peptide hormones) biosynthesis at the lesion site — often genes encoding phytohormone biosynthesis enzymes are present in the parasite genomes themselves.

Spontaneous tumor formation is a much rarer phenomenon; it is noted in some interspecific hybrids and inbred lines (Ahuja, 1998). In *Arabidopsis thaliana* several monogenic mutations leading to the development of tumors were experimentally produced (Frank et al., 2002; Krupkova et al., 2007) — in most cases, these mutations caused a decrease in cell adhesion, as a result of defects in the synthesis of cell wall components. The causes of spontaneous tumor formation in the plant interspecific hybrids and inbred lines are much less understood. It is believed that tumor formation in them probably depends on changes in the expression levels

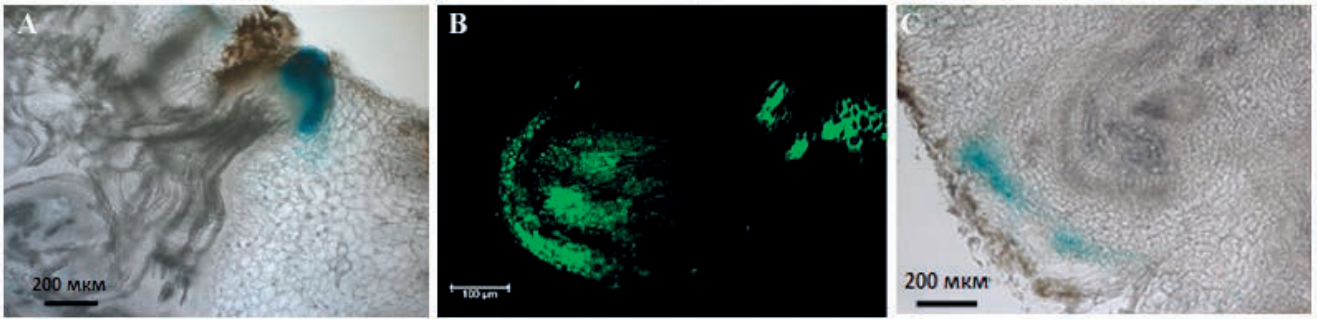

Fig. 3. Localization of the activity of promoters of genes encoding components of WOX-CLAVATA system in radish spontaneous tumors. A — *PWOX5::GUS*, B — *PCLV2::GFP*, C — *PCLE41::GUS.*

of many genes of different groups on a specific genetic background. In particular, in *Nicotiana* interspecific hybrids, tumor formation is presumably associated with the expression of sequences acquired by some tobacco species as a result of horizontal gene transfer from *Agrobacterium rhizogenes* (Intrieri and Buiatti, 2001), which may increase the sensitivity of plant tissues to endogenous cytokinins and auxins (Qu et al., 2006).

Spontaneous tumors in inbred lines and varieties were described in the mid-twentieth century for some plant species (see Ahuja, 1998 review), but data on the genetic control of most examples of plant tumor formation were not obtained. Obviously, in cross-pollinating species, prolonged inbreeding leads to homozygosis of recessive mutations, including mutations in genes associated with the control of cell division and differentiation.

Tumors in the crop-roots of radish inbred lines begin to develop when the plants pass to the generative stage of development and become noticeable during the flowering period (Buzovkina and Lutova, 2007). Externally and anatomically, they are similar to tumors induced by *Agrobacterium tumefaciens*, and are also capable of hormone-independent growth when isolated from the parent plant. According to histological analysis (Ilina et al., 2006; Lebedeva et al., 2015), these tumors are composed of a mass of small undifferentiated cells with separate foci of meristematic activity and a large number of abnormally oriented vessels. Upon detection of cell proliferation in the roots and tumors of inbred radish lines using the 5'-ethynyl-2-deoxyuridine intercalating dye (EdU), it was shown that during the formation of a spontaneous tumor, the proliferation of a group of interstitial cambium cells and the adjacent xylem parenchymal ray cell takes place, leading to the formation of an outgrowth consisting of undifferentiated actively dividing cells (Fig. 2). Interestingly, the activation of division of cambium and xylem parenchyma cells during tumor induction does not begin along the entire length of these zones, but in certain sites opposite the protoxylem pole, which suggests the heterogeneity of the cells of the cambial ring in the storage root of radish. Indeed, it is known that the interstitial cambium is laid from the pericycle cells; however, it is the pericycle cells opposite the xylem pole (the so-called "xylem pericycle") that retain undifferentiated status for a long time, and these cells and their descendants are used to induce other types of meristem: primordia of lateral roots, nodules, callus (Dodueva et al., 2014). Probably, induction of spontaneous tumors in radish lines is associated with this function of the most pluripotent, but not long-lived root meristem, the pericycle. At later stages, the conductive system of the tumor is formed, eventually mature spontaneous tumors contain a large number of abnormally oriented vessels connected to the root vascular system, to which few proliferating small cells with dense cytoplasm and thin cell walls adjoining cambium cells are attached. The bulk of proliferating cells is concentrated on the periphery of the tumor, at the points where the vascular bundles terminate, and has the appearance of organized structures morphologically similar to the apical meristems (Lebedeva et al., 2015), and the expression of certain meristem-specific genes is concentrated in these meristem-like zones (see below).

Genetic analysis showed that spontaneous tumor formation in radish is a polygenic trait; however, in some hybrid combinations it is inherited as a monogenic recessive trait (Matveeva et al., 2000, 2004). Despite the long-term study of spontaneous tumors in the radish inbred lines, the exact mechanisms of their development has not been identified; however, a number of interesting data on the participation of genes from different groups in tumor growth in radishes was obtained. Thus, spontaneous tumor formation in the lines of the genetic collection of radishes is currently one of the few examples of using plant tumor growth as a model for studying the systemic control of cell division.

The most likely cause of tumor formation in radish inbred lines is a change in the balance of cytokinins and auxins in root tissues. Thus, it was shown that the

content of cytokinins, especially free zeatin, in the tissues of the tumorous radish lines is several times higher than that of the related non-tumorous lines. In this case, a sharp increase in the level of cytokinins in the roots of tumor lines occurs during the beginning of flowering, that is, coincides with the induction of tumor formation; at the same time, during tumor formation, a decrease in IAA concentration in the root crop is observed (Matveeva et al., 2004; Dodueva et al., 2008). Transformation of the non-tumorous radish lines by the cytokinine biosynthesis gene *ipt* of *A. tumefaciens* under the constitutive promoter also led to tumor formation (Frolova et al., 2004). Finally, activation of the expression of cytokinin biosynthesis genes (*IPT* and *LOG* family), auxin biosynthesis genes (*YUC* family) and cytokinin primary response genes, such as *ARR5* (Lutova et al., 2008), was found at the root of tumorous radish lines during the flowering period.

One of the original ideas proposed by our research team in the study of spontaneous tumors of radish inbred lines was the assumption of the meristem nature of the plant tumors. This assumption was led by our data on the activation of the meristem-specific genes expression, including the genes encoding homeodomain-containing transcription factors (TF), during tumor development (Lutova et al., 2008; Tvorogova et al. 2012). Homeodomain-containing TFs control the *de novo* meristems formation, the maintenance of stem cell pools in them, the balance of cell proliferation and differentiation in meristems, as well as the metabolism and signal transduction of phytohormones. The central role in these processes is played by two groups of homeodomain-containing TF: KNOX (KNOTTED1-Related Homeobox) and WOX (WUSCHEL-Related Homeobox). The functions of TF KNOX are known to be associated with the control of cell proliferation mainly in shoot meristems (Tsuda and Hake, 2015), while TF WOX is necessary for maintaining stem cells in undifferentiated status (Dolzblasz et al., 2016). The literature describes in detail the role of TF KNOX and WOX in the development of the main plant meristems — the shoot and root apical meristem and also the lateral meristem cambium (reviewed in Lutova et al., 2015); in recent years, data have been obtained on the role of these TFs in the development of some types of irregular secondary meristem (Osipova et al., 2012; Azarakhsh et al., 2015).

The genes *KNAT1* from the *KNOX* family and *WOX5* belonging to the *WOX* family are meristem-specific genes whose expression is detected in spontaneous tumors in radish lines (Lutova et al., 2008; Tvorogova et al., 2013; Lebedeva et al., 2015). According to the literature, homologs of *KNAT1* are regulators of the shoot apical meristem (Reiser et al., 2000), cambium (Liebsch et al., 2014) and nodules (Azarakhsh et al., 2015). The *WOX5* gene is the central regulator of root apical meristem (Sarkar et al., 2007), and according to our data, it also regulates the development of symbiotic nodules in legumes (Osipova et al., 2012). Local analysis of the expression of these genes in the crop-roots and spontaneous tumors of radish lines using reporter constructs carrying the glucuronidase (*GUS*) showed different spatial expression patterns (Lebedeva et al., 2015; Lutova et al., 2015). Before the initiation of tumors, the expression of both genes takes place in the cambium — in the case of *WOX5*, it is confined to the part of cambium opposite the proto-xylem pole. In the tumors, *WOX5* promoter activity is concentrated in several "foci" inside the tumor (possibly corresponding to hypothetical organizing centers of the tumor, similarly to organizing centers of the apical meristem), while the activity of the *KNAT1* promoter is observed throughout the entire tumor area.

It is possible that activation of expression of genes encoding meristem regulators during tumor formation leads to a previously identified "shift" in the balance of phytohormones in tumor tissues: according to literature data, the direct targets of the KNOX TF include the cytokinin biosynthesis genes *IPT* and *LOG* (Jasinski et al., 2005; Bolduc and Hake, 2012), while the targets of negative regulation of TF WOX are A-type *ARR* genes encoding repressors of response to cytokinin (Leibfreid et al., 2005). Thus, the development of normal and abnormal meristem has a similar mechanism, depending on the activity of TF KNOX and WOX, which probably cause an increase in cytokinin levels in the tissues and also a cellular response to them.

It is possible that other regulators are also involved in controlling the development of abnormal meristems — tumors, including those interacting with TF KNOX and WOX. We obtained data on the possible participation of the components of the CLAVATA system, which regulate the expression levels of *WOX* genes in normal meristems, in tumor formation in radish inbred lines. The CLAVATA systems, first identified in the shoot apical meristem (Laux et al., 1996), include short CLE (CLAVATA3 / ENDOSPERM SURROUNDING REGION) signal peptides and their receptors, CLAVATA1-like Ser-Thr kinases with leucine-rich repeats. By binding to their receptors, CLE peptides trigger a poorly understood signaling pathway that regulates the expression of *WOX* genes (in most cases, negatively) (Dolzblasz et al., 2016). Thus, CLE peptides, their receptors and *WOX* genes form the so-called WOX–CLAVATA systems, conservative regulatory modules that control the activity of different meristems and maintain stem cell pools in them (reviewed in Dodueva et al., 2017). CLE peptides are very diverse (for example, 32 CLE genes are present in the *Arabidopsis* genome), two functionally different groups are distinguished among them — the numerous CLE peptides of group A limit the size of the meristem, negatively regulating the expression of *WOX*

genes; while CLE peptides of small group B, which work mainly in cambiums, activate the expression of their target *WOX4* genes, increasing cambium area (Ito et al., 2006; Whitford et al., 2008).

Literary data on the role of CLE peptides in the development of plant tumors are mainly represented by data on the role of these peptides in the development of root galls caused by parasitic nematodes: some nematodes have *CLE* genes, possibly derived from plants by horizontal transfer. CLE-peptides of parasitic nematodes are part of the secretion of salivary glands injected into the root tissue; on the surface of plant cells, they bind to plant CLAVATA1-like receptors and activate an unexplored signaling pathway leading to abnormal tissue proliferation (Kyohara et al., 2012).

In radishes, an analysis of *CLE* genes expression during the development of spontaneous tumors showed a manifold increase in the relative expression levels of eight *CLE* genes. Among them are *CLE19* and *CLE41*, which according to our data also play a central role in cambium proliferation and xylem differentiation in the development of the radish crop-root (Gancheva et al., 2016, 2018). The activity of *CLE19* and *CLE41* promoters in spontaneous radish tumors was also confirmed using reporter constructs, while the *CLE41* promoter activity was detected in the meristem-like zones of tumor, and the *CLE19* promoter was active in the xylem parenchyma and young vessels (Kuznetsova et al., 2018). In addition, an increase of *CLE41* gene expression during tumor growth was detected by analyzing transcriptomes of spontaneous tumors, as well as tumors induced by *A. tumefaciens* (Tkachenko et al., 2016). Finally, in the experiments on overexpression of *CLE19* and *CLE41* in the roots of radish and its wild ancestor *Raphanus raphanistrum*, it was shown that *CLE41* overexpression in *R. raphanistrum* not only activates the proliferation of cambium cells, but also causes the development of tumors on the root and lower parts of the stem, which are very similar to spontaneous tumors in the *R. sativus* inbred lines (Gancheva et al., 2016, 2018). Thus, among the CLE peptides, candidates for the role of conservative regulators of the development of normal meristem (procambium and cambium) as well as abnormal meristems (tumors) were identified: these are peptides CLE19 and CLE41. The search for probable targets of its action in the development of the root, as well as the search for CLE peptide receptors working in spontaneous tumors of radish lines and tumors induced by *A. tumefaciens*, is currently underway. The first data on the localization of the CLV2 receptor on the membranes of radish tumor cells were obtained (Malovichko et al., 2017).

Considering the previously reported hormonal nature of tumors in radish (Matveeva et al., 2004), we studied the possible relationship of CLE peptides with the auxin–cytokinin system. Analysis of the expression of identified radish *CLE* genes in radish seedlings in response to different periods of treatment with exogenous cytokinins and auxins showed that cytokinins suppress the expression of *CLE* genes encoding peptides of group A (negative regulators of meristem maintenance) and do not affect the expression of *CLE* genes of group B (cambium proliferation activators). At the same time, auxins suppress the expression of *CLE* genes of group B and activate the expression of some *CLE* genes of group A, such as *CLE19* (Dodueva et al., 2013; Gancheva et al., 2016). Thus, the action of auxins and cytokinins in the development of spontaneous tumors in radish lines can be mediated through the WOX-CLAVATA system, of which CLE peptides are a component.

Thus, the study of tumors in higher plants, namely spontaneous tumors in the radish inbred lines, allowed us for the first time to demonstrate the meristem-like nature of the tumors in higher plants, as well as to identify a number of common key regulators of the development of normal and abnormal meristems: they include genes encoding the homeodomain-containing TF of the KNOX and WOX families, as well as CLE peptides.

Conclusion

Identification of the role of WOX-CLAVATA systems, as well as other meristem-specific TF in the development of irregular meristem and neoplasm in higher plants has been the central topic of our research team in recent years; a number of priority results were obtained in this direction (Osipova et al., 2012; Azarakhsh et al., 2015; Tvorogova et al., 2018a), including data on the meristem-like nature of plant tumors of different origin and the participation of meristem-specific TF in their development (Lebedeva et al., 2015; Paponova et al., 2017; Samorodova et al., 2018).

Detection of differences between tumorous and non-tumorous radish lines for the in vitro regeneration features made it possible to draw parallels between the morphogenesis of intact plants and cultivated explants and to suggest that these signs may be under a single genetic control (Lutova et al., 1994, 2008). These assumptions were confirmed in various plant species — a clear example is the identification of common mechanisms of somatic and zygotic embryogenesis in higher plants based on data obtained by members of the Department of Genetics and Biotechnology of St. Petersburg State University on embryogenic lines of alfalfa, *Medicago truncatula* (Tvorogova et al., 2018a, 2018b).

Thus, using the traditional way from phenotype to genotype, on the models of the genetic collection of radish inbred lines differing in growth and development processes, we studied the genetic control of the regeneration processes in plants. Summarizing the results obtained in recent years using the genetic collection of

radish, it can be said that this model allowed us to obtain a number of new interesting data on de novo meristem formation and tumor growth, and to reveal a number of conservative regulators of these processes.

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